Effects of α-Tocopherol and β-Carotene Supplementation on Upper Aerodigestive Tract Cancers in a Large, Randomized Controlled Trial

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BACKGROUND. Although smoking and alcohol consumption are the major risk factors for upper aerodigestive tract cancers, observational studies indicate a protective role for fruits, vegetables, and antioxidant nutrients.

METHODS. The authors examined whether daily supplementation with 50 mg dl α-tocopheryl acetate and/or 20 mg β-carotene reduced the incidence of or mortality from oral/pharyngeal, esophageal, and laryngeal cancers in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) study, a double-blind, placebo-controlled primary prevention trial conducted in southwestern Finland. A total of 29,133 male smokers, aged 50–69 years and free of cancer at baseline, were randomized in a 2 × 2 factorial design to the supplementation regimen for 5–8 years (median, 6.1 years). Incident cancers of the oral cavity and pharynx (n = 65), esophagus (n = 24), and larynx (n = 56) were identified through the Finnish Cancer Registry. Intervention effects were assessed using survival analysis and proportional hazards models.

RESULTS. There was no effect of either agent on the overall incidence of any upper aerodigestive tract cancer. For larynx, however, exploratory subgroup analyses were suggestive of a protective effect of β-carotene supplementation on the incidence of early stage malignancies (stage I, relative risk [RR], 0.28, 95% confidence interval [CI]: 0.10–0.75). Neither agent affected mortality from these neoplasms.

CONCLUSIONS. The results do not provide support for a protective effect of vitamin E or β-carotene supplementation on upper aerodigestive tract cancers, although β-carotene supplementation may impact the incidence of some subtypes of laryngeal tumors. ***Cancer* 2007;109:891–8.**

KEYWORDS: β-carotene, chemoprevention, randomized trial, α-tocopherol, upper aerodigestive tract cancers.

Cancers of the upper aerodigestive tract, which include those arising in the oral cavity and pharynx, esophagus, and larynx, are a significant cause of morbidity and mortality throughout the world. Over 308,000 oral/pharyngeal cancers, 462,000 esophageal cancers, and 159,000 laryngeal cancers were estimated to have occurred globally in 2002, with incidence rates substantially higher in men than in women at all sites.[1] Overall 5-year survival rates for head and neck cancer—including oral/pharyngeal and laryngeal tumors—are approximately 50%, although local recurrences and second primary tumors are common.[2] In contrast, 5-year survival for esophageal cancer is only 15%.[3] Because population-based screening approaches are not currently available for these cancers,

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primary prevention remains the best way to reduce the health burdens attributable to these malignancies.

Tobacco smoking and alcohol consumption are the predominant risk factors for squamous cell carcinomas of the upper aerodigestive tract.[4] Both risk factors exert their carcinogenic effects in large part through oxidative mechanisms; accordingly, increased consumption of antioxidant-rich fruits and vegetables, as well as higher dietary intakes and blood levels of individual antioxidant nutrients (particularly β-carotene), have been associated with a reduced risk of upper aerodigestive tract cancers in several observational studies.[5] Further evidence for a protective effect of antioxidant nutrients against these cancers has been obtained from several small, short-term supplementation trials that demonstrated substantial benefit against premalignant endpoints and overt malignancy in high-risk individuals.[6]

We examined whether daily supplementation with α-tocopherol and/or β-carotene for 5–8 years reduces the incidence of and mortality from site-specific upper aerodigestive tract cancers in male smokers participating in a large, randomized, controlled chemoprevention trial in Finland.

MATERIALS AND METHODS

Study Population

The Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) study was a randomized, double-blind, placebo-controlled, primary prevention trial that tested whether daily supplementation with β-carotene (20 mg) and/or vitamin E (50 mg dl α-tocopheryl acetate) reduced the incidence of lung and other cancers. Details regarding study design, methods, participant characteristics, and compliance have been reported previously,[7] as have the main trial findings for selected cancers.[8] Briefly, 29,133 participants meeting all eligibility criteria at entry (male residents of southwestern Finland aged 50–69 years who smoked 5 or more cigarettes per day) were successfully randomized within 1 of 14 study centers into the trial between 1985 and 1988. Participants were allocated to 1 of 4 treatment groups (α-tocopherol + β-carotene, α-tocopherol alone, β-carotene alone, or a placebo) in a 2 × 2 factorial design, which enabled independent assessment of the intervention agents. Reasons for exclusion included a history of cancer (other than nonmelanoma skin cancer or carcinoma in situ), severe angina on exertion, chronic renal insufficiency, liver cirrhosis, chronic alcoholism, other diseases/conditions that might limit participation in a long-term intervention trial, receiving anticoagulant therapy, or refusal to discontinue use of vitamin E, vitamin A, or β-carotene supplements in excess of predefined amounts (>20 mg vitamin E, >20,000 IU vitamin A, >6 mg β-carotene). The trial ended on April 30, 1993 after 5–8 years of active intervention (median, 6.1 years). The Institutional Review Boards of both the National Public Health Institute of Finland and the US National Cancer Institute approved the study and written, informed consent was obtained from each participant before randomization. An independent Data and Safety Monitoring Committee convened twice a year throughout the trial to review study progress, potential side effects, and efficacy.

Endpoint Ascertainment

Incident cancers of the oral cavity and pharynx (ICD-9 codes 140–149), esophagus (ICD-9 code 150), and larynx (ICD-9 code 161) were identified through the Finnish Cancer Registry, which provides close to 100% case ascertainment nationwide,[9] and through death certificates. Upon identification of a case, all relevant medical records were obtained and reviewed independently by 1 or 2 study physicians. Pathology and cytology specimens were evaluated by 1 or 2 pathologists. Information regarding histology type and anatomic sublocation of disease was available for each cancer site, whereas tumor stage (based on the 1992 American Joint Committee on Cancer TNM classification system[10]) was only available for laryngeal carcinomas. For 1 participant with 2 separate oral/pharyngeal cancers, only the 1 that was diagnosed first was used in this analysis. All deaths were ascertained through the Register of Causes of Death.

Data Collection

Before randomization, all subjects were asked to provide detailed demographic, smoking, and occupational information, to give a history of medical examinations and physician-confirmed diseases, and to complete a dietary questionnaire. The food use questionnaire inquired about the usual frequency of consumption and portion sizes of 276 common food items / mixed dishes and beverages during the past year.[11] A color picture booklet was provided to each participant in order to assist with portion size estimation. Daily nutrient intakes, including values for vitamin E, β-carotene, and alcohol, were calculated using the food composition database of the National Public Health Institute in Finland. A total of 27,111 participants (93%) had complete dietary information available for analysis. An overnight fasting blood sample was collected from each participant at baseline, protected from light, divided into aliquots, and stored

at −70°C until analyzed. Concentrations of α-tocopherol, β-carotene, and retinol were determined using high-performance liquid chromatography (HPLC),[12] whereas total and high-density lipoprotein (HDL) cholesterol levels were measured using an enzymatic assay (CHOD-PAP method, Boehringer Mannheim, Germany).

Statistical Analysis

All statistical analyses were based on the 'intention-to-treat' principle in which individuals are analyzed as randomized, regardless of adherence to the allocated intervention. Person-years of observation were calculated for site-specific upper aerodigestive tract cancers from the date of randomization to the date of pertinent diagnosis, death, or trial closure. Kaplan-Meier cumulative incidence was plotted for the 4 intervention groups and for the 2 agents separately. The unweighted log-rank statistic and corresponding 2-sided *P*-value was utilized to test for significant differences in incidence and survival between the intervention groups. Relative risks (RRs) and 95% confidence intervals (CIs) for the intervention groups (α-tocopherol, α-tocopherol + β-carotene, and β-carotene, all vs placebo), as well as each individual agent (α-tocopherol vs no α-tocopherol and β-carotene vs no β-carotene), were estimated with Cox proportional hazards models. Effect modification by baseline factors (each split at the median value based on the distribution in the entire cohort), including dietary and serum α-tocopherol and β-carotene, smoking dose and duration, and alcohol consumption, was evaluated in stratified analyses and by adding the relevant cross-product term to main effects models. Differences in risk according to follow-up year of diagnosis, anatomic sublocation of disease, histology type, and stage, when available, were assessed for each cancer site in stratified analyses and with the chi-square test.

Associations of baseline dietary intakes of α-tocopherol and β-carotene, as well as serum levels of these nutrients, with upper aerodigestive tract cancers were assessed in multivariate models adjusted for intervention assignment, age at randomization, smoking dose and duration, alcohol consumption, body mass index (BMI), and education level. All models that included dietary variables were additionally adjusted for energy intake via the residual method,[13] whereas models with serum variables were further adjusted for serum cholesterol levels. Tests for linear trend were carried out by taking the median value of each nutrient tertile to model this exposure as a continuous variable. All reported *P*-values are 2-sided. Statistical analyses were performed using SAS software v. 8.02 (SAS Institute, Cary, NC).

TABLE 1
Baseline Characteristics (Medians and Proportions) of Trial Participants According to Intervention Group

	Intervention group			
	AT	AT + BC	BC	Placebo
No. of subjects	7286	7278	7282	7287
Age, y	57.2	57.3	57.2	56.9
BMI, kg/m^2	26.0	26.0	25.9	26.0
>Primary school education, %				
Cigarettes/day	20	20	20	20
Years of smoking	36	37	37	36
Serum α-tocopherol, mg/L	11.5	11.6	11.5	11.5
Serum β-carotene, μg/L	168	172	170	171
Serum cholesterol, mmol/L	6.15	6.18	6.15	6.14
Dietary intake, daily				
Energy, kcal	2736	2714	2721	2710
Fat, g	118	117	118	116
Fruits and vegetables, g	206	208	207	208
Vitamin E, mg	10.7	10.8	10.8	10.6
β-carotene, μg	1694	1727	1702	1723
Alcohol, g	11.3	10.8	11.0	10.8

BMI indicates body mass index; BC, β-carotene; AT, α-tocopherol.

RESULTS

Baseline characteristics, including age, smoking, dietary factors, and serum nutrients, were well-balanced across the 4 intervention groups (Table 1). Capsule compliance was also similar across the groups, with 4 out of 5 participants taking more than 95% of their capsules during the trial. Among men supplemented with α-tocopherol, serum concentrations of this nutrient increased from 11.5 mg/L at baseline to 17.4 mg/L after 3 years of active intervention, with corresponding values in those not receiving α-tocopherol being 11.5 mg/L and 12.4 mg/L, respectively. Baseline β-carotene levels increased substantially in the same interval among participants randomized to the β-carotene supplement (from 171 μg/L to 2954 μg/L), but remained relatively constant among those who did not receive β-carotene (from 170 μg/L to 180 μg/L).

During the trial period, 65 oral/pharyngeal, 24 esophageal, and 56 laryngeal cancers were diagnosed, with squamous cell carcinomas accounting for 94%, 71%, and 98% of cases, respectively. RRs and 95% CIs for the incidence of each site-specific upper aerodigestive tract cancer according to the 4 treatment groups and 2 intervention agents are shown in Table 2. Overall, there were no statistically

TABLE 2
Relative Risks for Incidence of and Mortality From Site-Specific Upper Aerodigestive Tract Cancers According to Trial Intervention Group*

	AT	AT + BC	BC	Placebo	AT	No AT	BC	No BC
Oral cavity/pharynx								
Incident cases, no.	15	17	15	18	32	33	32	33
RR (95% CI)	0.84 (0.42–1.66)	0.95 (0.49–1.84)	0.84 (0.42–1.66)	1.00	0.97 (0.60–1.58)	1.00	0.97 (0.60–1.58)	1.00
Deaths, no.	5	6	4	2	11	6	10	7
RR (95% CI)	2.51 (0.49–12.92)	3.01 (0.61–14.93)	2.01 (0.37–10.95)	1.00	1.84 (0.68–4.97)	1.00	1.43 (0.55–3.76)	1.00
Esophagus								
Incident cases, no.	6	5	6	7	11	13	11	13
RR (95% CI)	0.86 (0.29–2.56)	0.72 (0.23–2.27)	0.86 (0.29–2.56)	1.00	0.85 (0.38–1.89)	1.00	0.85 (0.38–1.90)	1.00
Deaths, no.	3	2	4	6	5	10	6	9
RR (95% CI)	0.50 (0.13–2.00)	0.34 (0.07–1.66)	0.67 (0.19–2.37)	1.00	0.50 (0.17–1.47)	1.00	0.67 (0.24–1.88)	1.00
Larynx								
Incident cases, no.	17	10	12	17	27	29	22	34
RR (95% CI)	1.00 (0.51–1.97)	0.59 (0.27–1.29)	0.71 (0.34–1.48)	1.00	0.93 (0.55–1.58)	1.00	0.65 (0.38–1.11)	1.00
Deaths, no.	2	2	3	3	4	6	5	5
RR (95% CI)	0.67 (0.11–4.00)	0.67 (0.11–4.00)	1.00 (0.20–4.96)	1.00	0.67 (0.19–2.37)	1.00	1.01 (0.29–3.46)	1.00

BC indicates β-carotene; CI, confidence interval; RR, relative risk; AT, α-tocopherol.

* Classified according to the 4 intervention groups or yes/no receipt of each specific intervention agent.

significant effects of either micronutrient on these cancer sites. There were no interactions between the α-tocopherol and β-carotene supplements for any of the cancer sites (all $P > .05$).

The cumulative incidence of each cancer was similar between men receiving and not receiving the α-tocopherol supplement, and all log-rank *P*-values exceeded .05 (Fig. 1). β-Carotene supplementation did not affect the cumulative incidence of oral/pharyngeal or esophageal cancer, but appeared to modestly, although not significantly, reduce the incidence of laryngeal cancers within 2 years of randomization (log-rank $P = .11$) (Fig. 1).

Because β-carotene supplementation marginally reduced the incidence of laryngeal cancer, we explored the effect within subgroups of several potentially important modifiers (Table 3). These analyses suggested that the β-carotene supplement may have been more protective against stage I laryngeal cancers (P interaction = .04), as well as those diagnosed during the first 2 years of intervention or localized to the glottis, and in participants who entered the trial with high serum β-carotene concentrations (P interaction $>.05$). There was no modification of the β-carotene effect by smoking intensity, alcohol intake, or serum α-tocopherol.

A total of 17 oral/pharyngeal, 15 esophageal, and 10 laryngeal cancer deaths occurred during the trial. Mortality for each cancer site was similar across the 4 treatment groups and for the 2 intervention agents (Table 2). Neither supplement affected survival time for any of the sites.

Baseline dietary intakes and serum levels of antioxidant nutrients were not significantly associated with oral/pharyngeal cancers (data not shown). Serum, but not dietary, β-carotene was inversely associated with esophageal cancer risk (for highest vs lowest tertile, RR = 0.07, 95% CI: 0.009–0.59, P trend = .008). Higher dietary intakes of α-tocopherol, β-carotene, and fruits and vegetables, as well as increased serum α-tocopherol concentrations, were associated with a lower risk of laryngeal cancer (all RR <0.50, all 95% CI exclude unity, all *P*-values for trend $<.05$). For example, the RR (95% CI) among individuals in the highest vs lowest tertile of dietary β-carotene intake was 0.35 (0.16–0.79), with a significant dose-response trend ($P = .01$); serum β-carotene concentrations were inversely, although not statistically significantly, associated with risk (for highest vs lowest tertile, RR = 0.81, 95% CI: 0.39–1.69, P trend = .61). The RR and 95% CI for laryngeal cancer according to increasing tertiles of fruit and vegetable intake were 1.00 (referent), 0.73 (0.39–1.36), and 0.36 (0.16–0.82), respectively (P trend = .01).

DISCUSSION

In this large primary prevention trial, we observed no overall effect of long-term α-tocopherol and/or β-carotene supplementation on the incidence of or mortality from oral/pharyngeal, esophageal, and laryngeal cancers. The β-carotene supplement, however, may have had some benefit against early-stage laryngeal cancers.

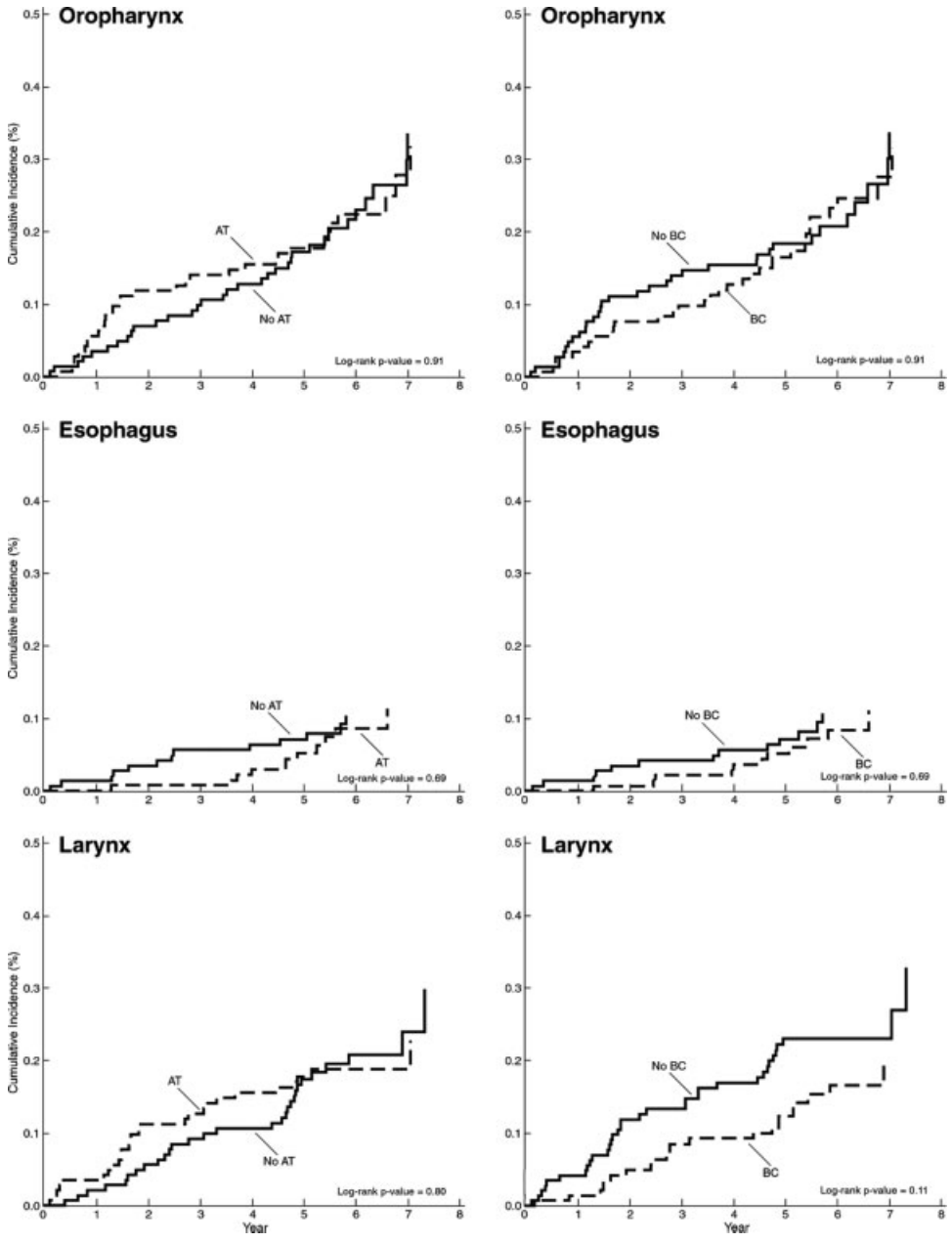

FIGURE 1. Kaplan-Meier cumulative incidence of cancers of the oral cavity and pharynx, esophagus, and larynx among participants who did and did not receive α-tocopherol (AT) and β-carotene (BC) supplementation.

One of 2 primary prevention trials conducted in Linxian, China—a region with 1 of the highest rates of esophageal/gastric cardia cancer in the world—showed that daily supplementation with a combination of β-carotene (15 mg), α-tocopherol (30 mg), and selenium (50 μg) for approximately 5 years significantly reduced the incidence of gastric cancer, as well as total, overall cancer, and gastric cancer mortality.[14] A second trial conducted among adults with esophageal dysplasia tested a supplement containing 14 vitamins and 12 minerals (including 15 mg β-carotene and 60 IU α-tocopherol) against a placebo and found no effect on incidence or mortality over 6 years, although nonsignificant reductions in esophageal cancer deaths were apparent.[15] Three controlled trials tested whether antioxidant supplementation reduced the incidence of second primary cancers and/or local recurrences in head and neck cancer patients. In the Carotene Prevention Trial, daily supplementation with 50 mg β-carotene for an average of 51 months had no statistically significant effect on local recurrence plus second primary head and neck cancers (larynx being the majority) in patients curatively treated for these tumors, although associations were inverse.[16] Similar to our findings, this trial observed discordant effects of β-carotene supple-

TABLE 3
Relative Risks for Laryngeal Cancer According to α-Tocopherol and β-Carotene Intervention Assignment, Stratified by Selected Characteristics

	AT	No AT	AT vs No AT	BC	No BC	BC vs No BC
	No. of cases	No. of cases	RR (95% CI)	No. of cases	No. of cases	RR (95% CI)
Follow-up time, y						
≤ 2	16	8	1.95 (0.83–4.60)	7	17	0.32 (0.13–0.78)
> 2	11	21	0.53 (0.25–1.09)	15	17	0.89 (0.44–1.78)
Stage at diagnosis						
I	9	14	0.64 (0.28–1.49)	5	18	0.28 (0.10–0.75)
II	8	6	1.34 (0.46–3.85)	6	8	0.75 (0.26–2.16)
III	2	6	0.33 (0.07–1.66)	3	5	0.60 (0.14–2.52)
IV	8	3	2.67 (0.71–10.07)	8	3	2.68 (0.71–10.09)
Anatomic subsite*						
Glottis	15	17	0.89 (0.44–1.77)	10	22	0.46 (0.22–0.96)
Supraglottis	10	11	0.91 (0.39–2.15)	11	10	1.10 (0.47–2.60)
Cigarettes/day						
<20	5	6	0.85 (0.26–2.79)	5	6	0.81 (0.25–2.67)
≥20	19	20	0.95 (0.51–1.78)	14	25	0.57 (0.30–1.10)
Years of cigarette smoking						
<36	6	5	1.20 (0.37–3.92)	4	7	0.58 (0.17–1.98)
≥36	18	21	0.87 (0.46–1.63)	15	24	0.62 (0.33–1.19)
Daily alcohol intake, g						
≤11	12	14	0.87 (0.40–1.87)	9	17	0.53 (0.24–1.19)
>11	12	12	1.00 (0.45–2.27)	10	14	0.72 (0.32–1.63)
Serum α-tocopherol, mg/L						
≤11.6	19	16	1.19 (0.61–2.32)	12	23	0.53 (0.27–1.07)
>11.6	8	13	0.62 (0.26–1.49)	10	11	0.89 (0.38–2.10)
Serum β-carotene, μg/L						
≤170	12	14	0.86 (0.40–1.85)	12	14	0.88 (0.41–1.89)
>170	12	12	1.01 (0.45–2.25)	7	17	0.41 (0.17–0.99)

BC indicates β-carotene; CI, confidence interval; RR, relative risk; AT, α-tocopherol.
P-value for interaction <.05 for AT × follow-up time, AT × years of cigarette smoking, BC × stage at diagnosis, and BC × years of cigarette smoking.
* Information missing for 3 cases.

mentation on second primary head and neck vs lung cancers, with a statistically nonsignificant elevation in risk at the latter site. In an Italian trial conducted in patients with radically treated stage I-II squamous head and neck tumors, there was no benefit of β-carotene supplementation (75 mg daily over an average of nearly 5 years) on the incidence of second primary tumors or disease-free survival.[17] The third trial tested whether a combination of α-tocopherol (400 IU/day) and β-carotene (30 mg/day) reduced the incidence of second primary cancers in head and neck cancer patients undergoing radiation therapy.[18] The use of β-carotene was halted during the trial, such that 71% of the participants only received α-tocopherol. An unexpectedly higher risk of second primary cancers (predominantly of the lung and trachea) was observed in the treatment vs the placebo group, although the same group experienced nonsignificantly lower rates of second primary tumors after the trial.

Our finding that β-carotene supplementation afforded possible protection against some laryngeal cancers contrasts with the small, yet statistically significant, adverse effect on lung cancer incidence and overall mortality that was observed in the same trial.[8,19] Although it may seem, at first, that β-carotene should produce similar effects at 2 respiratory sites that share common risk factors (ie, cigarette smoking), it is possible that this micronutrient exhibits different biological functions in each organ. Trials using intermediate endpoints have demonstrated that supplemental β-carotene leads to regression of oral precancerous lesions[6] but has no effect on sputum atypia—a potential intermediate marker for lung cancer.[20] Data from animal studies support these findings, showing that β-carotene prevents buccal pouch carcinogenesis in hamsters but does not influence lung carcinogenesis in hamsters or mice.[21] It now appears that adverse effects in the lung may be due to increased formation of oxidative

metabolites of β-carotene and subsequent interference with normal retinoid signaling, as recently demonstrated in ferrets.[22,23] Alternatively, it could be speculated that underdiagnosis of small laryngeal tumors resulted from the increased incidence and diagnosis of lung cancers (or possible misclassification of subglottal tumors as tracheal in nature) in the β-carotene group.

We observed strong inverse associations between baseline dietary intakes of α-tocopherol and β-carotene, as well as serum levels of α-tocopherol, and risks of laryngeal cancer. Several other observational studies have examined associations between antioxidant nutrients and risk of upper aerodigestive tract cancers, although few have been prospective and most were constrained by small numbers of cases. In a nested case-control study conducted among Japanese-American men in Hawaii, a significantly lower risk of overall and site-specific upper aerodigestive tract cancers was observed with higher baseline serum levels of β-carotene.[24] A strong inverse association between serum β-carotene and risk of oral and pharyngeal cancers was also observed in the Washington County, Maryland cohort, with higher levels of α-tocopherol showing protective effects against oral cancer in later years.[25] In the Chinese General Population Trial, prerandomization serum β-carotene levels were not related to esophageal squamous cell carcinoma (ESCC), but higher serum α-tocopherol levels were significantly associated with lower risk of this cancer.[26,27] In contrast to the aforementioned findings, serum micronutrient levels were unrelated to risks of upper aerodigestive tract cancers in a Finnish nested case-control study,[28] as were higher dietary intakes of β-carotene and vitamin E in a cohort of women.[29]

The lack of overall benefit of β-carotene and α-tocopherol supplementation contrasts with the strong inverse findings for baseline dietary and serum antioxidants in relation to esophageal and laryngeal cancers. These discrepancies might have arisen because dietary antioxidants are likely to exert their protective effects through interactions with other vitamins and phytochemicals found in the same food sources[30]; supplements only contain large quantities of a single antioxidant nutrient and are therefore ingested without the added benefit of potentially important cofactors. Furthermore, pharmacologic doses of antioxidants may modulate different biological pathways than levels achieved through dietary means. In addition, supplementation trials occur later in life and for a relatively short period of time, whereas dietary intake is reflective of habitual consumption over many decades. Finally, observational studies are subject to residual confounding by known or unmeasured factors, whereas randomized trials are typically free of this.[31]

The anticarcinogenic properties of β-carotene and α-tocopherol are largely attributed to their powerful antioxidant activities. β-Carotene efficiently quenches singlet oxygen, whereas α-tocopherol is the primary fat-soluble chain-breaking antioxidant nutrient that protects membrane lipids from peroxidation.[32,33] These micronutrients also have several additional biological functions independent of their antioxidant activity. For example, β-carotene is a precursor of vitamin A, which is an essential nutrient required for proper epithelial cell growth and differentiation.[34] The observed protective effect of supplemental β-carotene on early-stage laryngeal cancers could be consistent with a growth-inhibitory effect of this micronutrient on small, organ-confined tumors. α-Tocopherol inhibits the activity of protein kinase C activity—an enzyme that plays an important role in proliferation, adhesion, and the immune response—and regulates the expression of several genes involved in growth, apoptosis, inflammation, and the antioxidant defense system.[33]

There are several notable strengths of this study. The randomization was successful and resulted in an even distribution of baseline characteristics across intervention groups, thereby eliminating confounding by measured and unknown factors. High capsule compliance, similar dropout rates across treatment groups, and complete follow-up also make bias an unlikely explanation for our findings. Limitations include the small number of site-specific cases available for analysis, which limited our power to detect modest associations, the fact that upper aerodigestive tract cancers were not the primary endpoints of the trial, and the possibility that our exploratory subgroup findings were due to chance. Finally, our findings may not be generalizable to racially and ethnically diverse populations, women, and nonsmokers.

In summary, neither α-tocopherol nor β-carotene supplementation decreased the risk of upper aerodigestive tract cancers in older male smokers, although modest reductions in the incidence of some laryngeal cancer subtypes were observed in men randomized to receive β-carotene. Given the adverse effects of β-carotene supplements on lung cancer incidence and overall mortality that have been observed in multiple trials, however, further investigation of supplemental β-carotene for prevention of laryngeal cancers in high-risk smokers cannot be justified. Instead, smokers should be encouraged to quit smoking and to consume adequate amounts of fruits and vegetables, which are a major source of antioxidant nutrients, including β-carotene. The latter is consistent with our findings of a significant inverse

association between laryngeal cancer and dietary intakes of both β-carotene and fruits and vegetables.

REFERENCES

1. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA Cancer J Clin*. 2005;55:74–108.
2. Papadimitrakopoulou VA. Chemoprevention of head and neck cancer: an update. *Curr Opin Oncol*. 2002;14:318–322.
3. Enzinger PC, Mayer RJ. Esophageal cancer. *N Engl J Med*. 2003;349:2241–2252.
4. Zeka A, Gore R, Kriebel D. Effects of alcohol and tobacco on aerodigestive cancer risks: a meta-regression analysis. *Cancer Causes Control*. 2003;14:897–906.
5. American Institute for Cancer Research, World Cancer Research Fund. Food, nutrition and the prevention of cancer: a global perspective. Washington, DC: American Institute for Cancer Research; 1997.
6. Mayne ST, Lippman SM. Cancer prevention: diet and chemopreventive agents. Retinoids, carotenoids, and micronutrients. In: DeVita VT, Hellman S, Rosenberg SA, editors. Principles & Practice of Oncology, 6th ed. Philadelphia: Lippincott Williams & Wilkins; 2001:575–590.
7. The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study Group. The alpha-tocopherol, beta-carotene lung cancer prevention study: design, methods, participant characteristics, and compliance. *Ann Epidemiol*. 1994;4:1–10.
8. The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study Group. The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. *N Engl J Med*. 1994;330:1029–1035.
9. Korhonen P, Malila N, Pukkala E, Teppo L, Albanes D, Virtamo J. The Finnish Cancer Registry as follow-up source of a large trial cohort—accuracy and delay. *Acta Oncol*. 2002;41:381–388.
10. Bears OH. Manual for Staging of Cancer, 4th ed. American Joint Committee on Cancer, American Cancer Society. Philadelphia: Lippincott; 1992.
11. Pietinen P, Hartman AM, Haapa E, et al. Reproducibility and validity of dietary assessment instruments. I. A self-administered food use questionnaire with a portion size picture booklet. *Am J Epidemiol*. 1988;128:655–666.
12. Milne DB, Botnen J. Retinol, alpha-tocopherol, lycopene, and alpha- and beta-carotene simultaneously determined in plasma by isocratic liquid chromatography. *Clin Chem*. 1986;32:874–876.
13. Willett W. Nutritional Epidemiology, 2nd ed. New York: Oxford University Press; 1998.
14. Blot WJ, Li JY, Taylor PR, et al. Nutrition intervention trials in Linxian, China: supplementation with specific vitamin/mineral combinations, cancer incidence, and disease-specific mortality in the general population. *J Natl Cancer Inst*. 1993;85:1483–1492.
15. Li JY, Taylor PR, Li B, et al. Nutrition intervention trials in Linxian, China: multiple vitamin/mineral supplementation, cancer incidence, and disease-specific mortality among adults with esophageal dysplasia. *J Natl Cancer Inst*. 1993;85:1492–1498.
16. Mayne ST, Cartmel B, Baum M, et al. Randomized trial of supplemental beta-carotene to prevent second head and neck cancer. *Cancer Res*. 2001;61:1457–1463.
17. Toma S, Bonelli L, Sartoris A, et al. Beta-carotene supplementation in patients radically treated for stage I-II head and neck cancer: results of a randomized trial. *Oncol Rep*. 2003;10:1895–1901.
18. Bairati I, Meyer F, Gelinas M, et al. A randomized trial of antioxidant vitamins to prevent second primary cancers in head and neck cancer patients. *J Natl Cancer Inst*. 2005;97:481–488.
19. Albanes D, Heinonen OP, Taylor PR, et al. Alpha-tocopherol and beta-carotene supplements and lung cancer incidence in the alpha-tocopherol, beta-carotene cancer prevention study: effects of base-line characteristics and study compliance. *J Natl Cancer Inst*. 1996;88:1560–1570.
20. McLarty JW, Holiday DB, Girard WM, Yanagihara RH, Kummet TD, Greenberg SD. Beta-carotene, vitamin A, and lung cancer chemoprevention: results of an intermediate endpoint study. *Am J Clin Nutr*. 1995;62(6 Suppl):1431S–1438S.
21. International Agency for Research on Cancer. IARC Handbooks of Cancer Prevention. Carotenoids. Vol. 2. Chapel Hill, NC: Oxford University Press; 1998.
22. Liu C, Russell RM, Wang XD. Exposing ferrets to cigarette smoke and a pharmacological dose of beta-carotene supplementation enhance in vitro retinoic acid catabolism in lungs via induction of cytochrome P450 enzymes. *J Nutr*. 2003;133:173–179.
23. Wang XD, Liu C, Bronson RT, Smith DE, Krinsky NI, Russell M. Retinoid signaling and activator protein-1 expression in ferrets given beta-carotene supplements and exposed to tobacco smoke. *J Natl Cancer Inst*. 1999;91:60–66.
24. Nomura AM, Ziegler RG, Stemmermann GN, Chyou PH, Craft NE. Serum micronutrients and upper aerodigestive tract cancer. *Cancer Epidemiol Biomarkers Prev*. 1997;6:407–412.
25. Zheng W, Blot WJ, Diamond EL, et al. Serum micronutrients and the subsequent risk of oral and pharyngeal cancer. *Cancer Res*. 1993;53:795–798.
26. Abnet CC, Qiao YL, Dawsey SM, et al. Prospective study of serum retinol, beta-carotene, beta-cryptoxanthin, and lutein/zeaxanthin and esophageal and gastric cancers in China. *Cancer Causes Control*. 2003;14:645–655.
27. Taylor PR, Qiao YL, Abnet CC, et al. Prospective study of serum vitamin E levels and esophageal and gastric cancers. *J Natl Cancer Inst*. 2003;95:1414–1416.
28. Knekt P, Aromaa A, Maatela J, et al. Serum micronutrients and risk of cancers of low incidence in Finland. *Am J Epidemiol*. 1991;134:356–361.
29. Kasum CM, Jacobs DR Jr, Nicodemus K, Folsom AR. Dietary risk factors for upper aerodigestive tract cancers. *Int J Cancer*. 2002;99:267–272.
30. Eastwood MA. Interaction of dietary antioxidants in vivo: how fruit and vegetables prevent disease? *QJM*. 1999;92:527–530.
31. Lawlor DA, Davey Smith G, Kundu D, Bruckdorfer KR, Ebrahim S. Those confounded vitamins: what can we learn from the differences between observational versus randomised trial evidence? *Lancet*. 2004;363:1724–1727.
32. Rock CL. Carotenoids: biology and treatment. *Pharmacol Ther*. 1997;75:185–197.
33. Ricciarelli R, Zingg JM, Azzi A. Vitamin E: protective role of a Janus molecule. *FASEB J*. 2001;15:2314–2325.
34. Bowman BA, Russell RM. International Life Sciences Institute-Nutrition Foundation. Present Knowledge in Nutrition, 8th ed. Washington, DC: ILSI Press International Life Sciences Institute; 2001.